

Attorney Docket No.49946-60261
Serial No.: 09/445,289
Applicants: Galina V. Mukamolova, *et al.*

Examiner: S. Devi
Group Art Unit: 1645

Amendments to the Specification:

Please amend the specification as follows:

Please delete the paragraph on page 40, line 4 and replace it with the following amended paragraph:

Part D. Motifs in the C-terminus (residues 158-322) of MtubMTVO43. **The A, B, C and D motifs are shown in SEQ ID NOS 62, 60, 29 and 61, respectively.**

Please delete the paragraph on page 48, lines 3-15, and replace it with the following amended paragraph:

Protein microsequence data from the N-terminus (**ATVDTWDRLAEexSNGTxD**) (**SEQ ID NO: 38**) and an internal peptide (**VGGE~~GYPHQASK~~**) (**SEQ ID NO: 42**) obtained from the purified RP-factor were used to design two oligonucleotides, denoted A1 [GCSACSGTSGACACSTGGGACCGSCTSGCSGAG] (**SEQ ID NO: 37**) and A2 [GCYTGRTGIGGRTAACCYTCICC] (**SEQ ID NO: 41**), respectively. Taq polymerase was employed under standard conditions to amplify a 147 bp PCR product from *M. luteus* DNA with these primers. The PCR product obtained from *M. luteus* DNA with these two primers was labelled with digoxigenin and used as a probe for Southern hybridisation experiments. *Sma*I-digested genomic DNA was size-fractionated by agarose gel electrophoresis and circa 1.4 kbp fragments were cloned in pMTL20 and established in *E. coli* strain DH5 α . Two recombinant plasmids carrying the desired insert were detected by hybridisation, confirmed by PCR using oligonucleotides A1 and A2, and one of them was manually sequenced on both strands using the dideoxy chain termination method.

Please delete the paragraph on page 51, lines 23-26 and replace it with the following amended paragraph:

Attorney Docket No.49946-60261
Serial No.: 09/445,289
Applicants: Galina V. Mukamolova, *et al.*

Examiner: S. Devi
Group Art Unit: 1645

Two acidic residues, D7 & E13 (numbering according to the *M. luteus* secreted protein), within this segment are absolutely conserved. The KAEQIKRAE segment (residues 51-59 of SEQ ID NO: 43) represents an island of particularly high surface probability. These elements may form part of functional domains within the RP-factor protein.

Please delete the section of text on page 51, lines 28-31 and replace it with the amended section of text:

The conserved domain contains four conserved tryptophan residues (one of which is in a region of high surface probability DTWDR - residues 4-8 of SEQ ID NO: 43). In the complex between human growth hormone and its first bound receptor, interactions involving two surface-located tryptophan residues in the receptor account for more than 75% of the binding

Please delete the section of text on page 54, lines 21-31, and replace it with the following amended section of text:

Two primers [5'-GTCAGAATTCATATGCCACCGTGGACACCTGG-3'] (SEQ ID NO: 46) and [5'-TGACGGATCCTATTAGGCCTGCAGGACGAG-3'] (SEQ ID NO: 47) were employed to amplify (5 cycles of 30s at 94°C, 30s at 60°C, 30s at 72°C, followed by 15 cycles of 30s at 94°C, 60s at 72°C) the RP-factor coding sequence (i.e. lacking the signal sequence) from the cloned 1.4 kbp *Sma*I fragment of genomic DNA. It was first established in *E. coli* DH5á as a 567 bp EcoRI-*Bam*HI fragment in pMTL20 and then excised as a 562 bp *Nde*I - *Bam*HI fragment, inserted into pET19b (Novagen) and re-established in *E. coli* DH5 á. The sequence of the PCR product and vector-insert junction in this plasmid, denoted pRPF 1, was verified. RP-factor was expressed from RPF 1 after transforming it into *E. coli* HSM174(DE3). The protein, containing a His₁₀ tag at the N-terminus, was isolated by sonicating bacteria, previously grown to an OD_{600nm}=0.6 and induced with

Attorney Docket No.49946-60261
Serial No.: 09/445,289
Applicants: Galina V. Mukamolova, *et al.*

Examiner: S. Devi
Group Art Unit: 1645

Please delete the section of text on page 56, lines 24-31, and replace it with the following amended section of text:

Two primers [5'-ATCAGAATTCATATGGACGACATCGATTGGGACGC-3'] (**SEQ ID NO: 48**) and [5'-CGCAGGATCCCTCAATCGTCCCTGCTCC-3'] (**SEQ ID NO: 49**) were employed to amplify (5 cycles of 30s at 94°C, 30s at 58°C, 30s at 72°C, followed by 25 cycles of 30s at 94°C, 60s at 72°C) the RP-factor coding sequence (i.e. lacking the signal sequence) from *M. tuberculosis* H37Rv genomic DNA. The PCR product was first established in *E. coli* DH5a as a 336 bp *Eco*RI-*Bam*HI fragment in pMTL20 and then excised as a 331 bp *Nde*I - *Bam*HI fragment, inserted into pET19b (Novagen) and re-established in *E. coli* DH5a. The sequence of the PCR product and vector-insert junction in this plasmid,

Please delete the section of text on page 59, lines 23-33 and replace it with the following amended section of text:

The entire *yabE* coding region together with flanking sequences was amplified from *B. subtilis* genomic DNA using primers D11 [5'-GAAGAGAATTCTCCATCACGA-3'] (**SEQ ID NO: 50**) and D12 [5'-CCAAACGAATTGGTCAATCAC-3'] (**SEQ ID NO: 51**) as a 1803 bp product. A 1186 bp *Hind*III-*Bc*II fragment encompassing the 3' end of the coding sequence was excised from the PCR product, ligated with *Hind*III + *Bam*HI-digested pMTL20, and used to transform *E. coli* strain DH5á with selection for ampicillin-resistance. Plasmid pYABE was isolated from one of the transformants. A 763 bp *Hind*III-*Bam*HI fragment from entirely within the *yabE* coding sequence was excised from the pYABE, ligated with *Hind*III + *Bam*HI-digested pMUTIN4, an integrating plasmid that may be employed for generating knockout mutations in *B. subtilis* (Edwards & Errington, 1997, Molecular Microbiology, 24, 905-915) and used to transform *E. coli* strain XL 1-Blue with coding sequence was excised from pYABE, ligated with *Hind*III + *Eco*RI digested pMUTIN4 and used to transform *E. coli* strain XL1-Blue with

Attorney Docket No.49946-60261
Serial No.: 09/445,289
Applicants: Galina V. Mukamolova, *et al.*

Examiner: S. Devi
Group Art Unit: 1645

Please delete the paragraph on page 60, lines 9-23, and replace it with the following amended paragraph:

The entire *yocH* coding region together with flanking sequences was amplified from *B. subtilis* genomic DNA using primers D10 [5'-GCAAGGATCCCAGACTAAAAAACAG-3'] (SEQ ID NO: 52) and D9 [5'-ATCAGGATCCATATTATTAGTTAAGA-3'] (SEQ ID NO: 53) as a 1145 bp product. A 358 bp *Hpa*I fragment from entirely within the *yocH* coding sequence was excised from the PCR product, ligated with *Sma*I-digested pMTL20, and used to transform *E. coli* strain XL 1-Blue with selection for ampicillin-resistance. Plasmid pYOC2a, containing an internal segment of the *yocH* coding sequence, was isolated from one of the transformants. The insert in this plasmid was then excised from pYOC2a as a 385 bp *Eco*RI-*Hind*III fragment and inserted into pMUTIN4, to yield pYOC2. A 307 bp *Hind*III-*Bam*HI fragment encompassing the 3' end of the *yocH* coding sequence was excised from the 1145 bp PCR product, ligated with *Hind*III + *Bam*HI digested pMUTIN4, and used to transform *E. coli* strain DH5á with selection for ampicillin-resistance. Plasmid pYOC3, containing a DNA segment encompassing the 3' end of the *yocH* coding sequence, was isolated from one of the transformants.

Please delete the current Sequence Listing and replace it with the substitute Sequence Listing (pages 1-31) submitted herewith, immediately following the last page of the application, and please renumber any pages of the application as needed.